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# BOTANICAL GAZETTE.

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Staining and Double Staining of Vegetable Tissues.—The increased attention given to vegetable histology, which is largely due to the cheapness of good microscopes, makes this a proper time to call attention to the recent improved modes of permanently mounting objects for study. Besides this the clinical microscopes now in use enable a lecturer to pass to his class the objects upon which he may be discoursing, with the different forms of tissue either in contrasting colors or in different shades of the same color.

It is simply historic justice to say that thus far Americans have been most successful in the preparation of these stained objects, and to none is more credit due than to Dr. J. G. Hunt, of Philadelphia, and to the late Dr. George D. Beatty, of Baltimore.

For convenience sake, we may divide the objects to be mounted into those which should first be bleached, and those which do not require such treatment.

Taking up the first class we may say it is such objects as are thicker than ordinary, or are of denser texture. Prominent among them we may name leaves and fronds, *when they are not cut in thin sections*, but are intended to be looked at through from surface to surface. *As a rule* such objects are better if they do not exceed half an inch in size; though one may succeed in bleaching much larger specimens, but it requires so long an immersion in the bleaching fluid that the texture of the plant is apt to be largely disintegrated, and consequently altered from its normal condition. I have now before me a specimen of the frond of *Asplenium Filix-fœmina*, R. Br., fully 2 inches long and one-third as wide, in which the frond texture (save the chlorophyll) is almost unaltered; the indusium, sporangium and spores all perfect and most beautifully stained. In such specimens bleaching is absolutely requisite. There are others in which it is simply a convenience, as for example in matured wood sections, where the processes of growth are ended and we no longer require the starch, protoplasm and chlorophyll. Here the bleaching removes these substances and leaves us the mere skeleton of parenchyma, fibres, ducts and the like, all cleared out and in better condition for clearer observation and hence better adapted to the use of a class.

I should here add that few, if any, fungi or lichens should be bleached; indeed they will almost certainly be injured or destroyed by it.

*Bleaching fluid:*—The best is Labarraque's Solution. The object is to be immersed in it and covered, for the double purpose of excluding dirt and of preventing the deterioration of the fluid by exposure to the air. How long it should be kept there depends on circumstances. The best rule is to say, until it is bleached, *but no longer.*

When it is bleached the object should be removed and placed in water, then in another water, then in water slightly acidulated with nitric acid, then into water and alcohol, half and half; after which it may be removed and placed in nearly pure alcohol where it is to remain until thoroughly translucent. If the object is of firm texture and not likely to be injured by the water, a day or two may be spent in the process of washing out all the Labarraque's solution, before it is finally assigned to the alcohol. If on the other hand (as in ferns where we desire to retain the sporangia and spores) it be more flimsy, the process must be hastened and should not take more than a couple of hours.

*Unbleached specimens.* These are *as a rule* cross or longitudinal sections, and in a general way we may say the thinner they are the better. It once was the custom to bleach these and under certain circumstances we may still do so. It will, however, remove the protoplasm, etc., and thus prevent any successful study of the action of the cell contents as related to growth and to cell-multiplication. I seldom bleach these now. Immediately that the section is made, I place it in a weak mixture of alcohol and water, then gradually increase the strength by adding alcohol. It is, however, to be observed that in the object as cut and before being placed in the alcohol we will find the protoplasm more or less diffused through the cell cavity, tending, however, to gather along the cell walls. The nucleus may be in the center or on one side. When, however, it has been soaked in alcohol, the protoplasm will be observed gathered into a more or less dense mass in the center and the nucleus will then resemble a nucleolus. This must be remembered in studying specimens which have been placed in alcohol. I do not regard any of the means advised to prevent this contraction of the protoplasm around the nucleus as entirely satisfactory.

The section is to be left in alcohol until it has become as clear as it can be made. How long this will be depends entirely on the character of the specimen and on the quantity of chlorophyll to be removed. As between the bleached and unbleached specimens, necessity may compel the bleaching, but never bleach when you can avoid it and still make a good specimen.

From this point the treatment of the bleached and unbleached specimens is the same, and the only question is as to the staining fluid: what one, or whether more than one. I give this as a general rule: if you are to use but one, let that one be the *Hæmatoxylin Dye.* My reasons are first, it is permanent; second, it is clear and beautiful; third, you can produce with it almost any shade from a deep, almost black-brown, to the slightest tinge of red; fourth, if by accident you obtain too deep a shade you may more satisfactorily remove part of it than of any other dye. This is done by placing the object in, say a tablespoonful of alcohol, and to this add a few drops of bichloride of tin. In a few minutes (often in a few seconds) the alcohol will be colored by the dye extracted and the object be by so much the lighter colored. It is best, however, not to allow the specimen to remain in too long, or it assumes a dead hue instead of the vivid color which it first assumed when immersed.

I give the formula I find in Dr. Beatty's article, republished from Science Gossip into the American Journal of Microscopy, June, 1876, and here would also state how much I am indebted to the article, both for my own methods of staining and for the facts of this contribution.

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| Take "Ground Campeachy Wood, | ½ ounce. |
| Pulv. Alum, | 1 ounce. |

Mix and triturate in a mortar for twenty minutes, then add five ounces of hot, distilled water and let it stand for two days. Filter, and to each ounce of the dye add two drachms of 75 per cent. alcohol. In twenty-four hours again filter to remove the precipitated alum."

I would add that all the dyes can be obtained from any respectable optician in our large cities and that it is cheaper to buy than to make them.

In using this, I take the requisite quantity of water to immerse the specimen and add to it just enough of the dye to give the shade I desire, then if but little of the dye was used I add a very little (say 4 per cent.) of powdered alum to the water. *This is not always or even usually necessary.* Then after mixing, for a moment, the dye and the the water I immerse the object in it. How long shall it stay there? Until it has done its work. Sometimes, as in some sections, this will be in a few minutes; in other cases it may be hours. Bear in mind, however, that the specimen is to be watched, lest owing to an affinity of certain portions of the plant structure for the dyes they attract enough (even in a weak solution) to make them black and opaque. Ordinarily the logwood dye is used alone, but there are cases, as in the fruiting fronds of ferns, where we may remove the specimen from the logwood and wash it hastily in a couple of waters to remove any alum crystals which may have formed on it, and then place it in pure alcohol (or nearly pure) which is just fairly tinged with iodine green (aniline), where it may remain for twenty-four hours or more and come out with the frond scarlet, the sporangium brown or brownish-black, and the spores bright grass-green. An object fit for ecstasy if one have a binocular microscope! If he do not have, we can only pity him.

It sometimes happens that plants which have a glaucous color do not bleach out so well as to stain evenly in logwood dye. I have found that a prolonged soaking in alcohol would usually remove the trouble.

Now as for the more usual double staining. Here one may have a choice of colors. I will for present purposes give only two, as the principles involved in the use of these would guide in the use of the others; and I will give those which in my own work have given me the greatest satisfaction.

Ammonia Carmine made after the formula of Dr. J. J. Woodward is:

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| "Pulv. Carmine, | 7½ | grains. |
| Water of Ammonia, | 20 | drops. |
| Absolute Alcohol, | ½ | ounce. |
| Glycerine, | 1 | ounce. |
| Distilled Water, | 1 | ounce. |

Put the pulverized carmine in a test tube and add the ammonia. Boil slowly for a few seconds and set aside, *uncorked*, for a day, to get rid of excess of ammonia. Add the mixed water and glycerine and next the alcohol; then filter."

The other dye I would indicate as the iodine-green. Of this I keep a strong alcoholic solution on my table. Let it be a dark green in strength and then it can be weakened to suit the purposes in each case.

Now for the method of using it. Taking the specimen, bleached or unbleached, from the alcohol where it has been "clearing up," I put it in fresh alcohol and to this add enough of the iodine-green solution to give a faint green tinge. Two or three drops in a tablespoonful will suffice ordinarily. This I set away for 12 to 24 hours as the case may be. Examining it after this lapse of time I find the alcohol has appreciably cleaned up. That is, portions of the plant structure have taken it from the solution. Moreover, and very important, only certain portions (say the bast) will have appropriated it, leaving the other free or almost free from any stain, white as when it left the Labarraque, almost. This then is an important point: the other anatomical elements are free to take another color without a dauby tinge which suggests bad workmanship.

I never am in a hurry to get it out of the iodine-green. Let it have time, and if required, add a few drops more of the dye. When, however, it is ready, I place down small porcelain saucers, or watch crystals, in the following order: to begin on my left hand, 1 water, 2 ammonia carmine, 3 alcohol, 4 alcohol, 5 alcohol, 6 *alcohol pure*, 7 oil of cloves. Then I take the specimen from the iodine-green, dip it a moment in water, then about 20 seconds to a minute in the carmine, then through the alcohols, in each of which it remains 10 to 20 minutes, except in the first where it remains only long enough to have the unfixed carmine washed away.

From the last alcohol (which should if possible be absolute) it goes into oil of cloves where it should remain long enough to become perfectly transparent. Five minutes may suffice for this, or it may require hours.

From oil of cloves I mount it permanently in *balsam*. If, however, any water remains in the specimen it will be found that it will cloud when it is placed in the balsam. Hence, as you value a nice, clear specimen, give it time in alcohol and in oil of cloves.

There are other ways than the one I have given to stain and double stain. But knowing the one, others will readily enough suggest themselves. Other colors too may be used, but these will do to start with. Particular objects may require particular dyes.

Thus on the same slide, I have 3 sections of *Peziza floccosa*. One is unstained and so transparent that it requires nice manipulation (in the balsam mount) to bring out either asci or spores. The next is stained with the hæmatoxylin dye and shows the hyphæ and the root-like hairs beautifully, but the asci and spores only tolerably. The third section is stained with a solution of iodine-green. This developes the spores and asci strikingly and leaves the hyphæ proper, uncolored.

Of course this illustration only points a general principle. I would, however, add that so far as my somewhat limited experience in making sections of fungi goes, I have found nothing which is more satisfactory for bringing out the spores than this iodine-green.

It may be well to say in conclusion that even the advanced investigator will find these stained slides of great service to him, as the dyes act so unerringly that often a stray cell or clump of cells of a different morphological character from the surrounding ones will be indicated at once. To the instructor they are of essential service. enabling him to say to his class:—the red is this and the green that. and so leave no possible ground for a misunderstanding.—

J. T. ROTHROCK.

EPIPACTIS HELLEBORINE, var. VIRIDENS (*E. viridiflora*, Reichenbach) a North American plant.—The Ladies' Botanical Club, of Syracuse, N. Y., has recently made a most interesting discovery, which I am desired to announce in the BOTANICAL GAZETTE. In a pine wood near Syracuse, early in August, one of the members of that spirited society, Mrs. Church, found this European *Epipactis*, growing along with *Pogonia pendula* and *Corallorhiza multiflora*. Two specimens only were collected, one of which was sent to me by Mrs. Rust, the president of the club; and on a second visit, the members of the club found three or four more specimens. The plant appears to be exactly the *E. viridiflora* of Reichenbach, well figured in the Iconographia Floræ Germanicæ, which, peculiar as it seems to be, is reduced by Irmisch to a variety of *E. Helleborine*. The type is not wholly new to North America, for *E. gigantea* is a California species, which also inhabits Texas. Orchids are the least introducible of plants. and I should have no doubt that this is truly indigenous in this only known American station. It is to be noted that it is found in the very district which is almost alone in possessing *Scolopendrium* (the indigenous character of which is confirmed by several localities, all in completely wild stations) also *Botrychium Lunaria*, a third European species of rare occurence in this country.—A. GRAY.

THE SYRACUSE BOTANICAL CLUB.—-Following the finding of *Epipactis Helleborine* it may be of interest to the readers of the GAZETTE to know something about the rise and progress of this energetic club composed entirely of ladies. The idea of a club seemed first to find its origin in a class organized for the purpose of studying Botany. This class was formed only last January and out of that start has grown a club that is doing good work and bids fair to verify the saying "by their fruits (and flowers) ye shall know them." The club was first called the Rust Botanical Club, but at the request of Mrs. Rust herself it was changed to its present name. The club meets weekly for work and study and monthly to transact business. The first study undertaken was that of their native ferns, a collection